EPA Reviewer: Lisa Austin, Ph.D. **Signature:**
Registration Action Branch 1, Health Effects Division (7509C) **Date:** 7/25/08
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TXR#: 0055057

DATA EVALUATION RECORD

STUDY TYPE: Metabolism - rat; OPPTS 870.7485 [§85-1)]; OECD 417.

PC CODE: 118203 **DP BARCODE:** D349929

TEST MATERIAL (PURITY): ^{14}C-BAS 800 H (chemical purity 100%, radiochemical purity 99.9%)

SYNONYMS: N'-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)-pyrimidinyl)benzoyl]-N-isopropyl-N-methylsulfamide-[phenyl-U- C^{14}]; BASF Reg. No. 4054449, saflufenacil

CITATION: Fabian, E. and Landsiedel, R. (2007) ^{14}C-BAS 800 H – Study on the Biokinetics in Rats. Experimental Toxicology and Ecology, BASF AG, Ludwigshafen, Germany, Project No. 02B0627/046008, 24 September 2007. BASF DocID 2007/1033929 17128129. Unpublished.

Grosshans, F. (2007) The Metabolism of ^{14}C-BAS 800 H (Reg.No, 4054449) in Rats. BASF Agricultural Center Limburgerhof, Germany, Study Code 132617, 17 October 2007. MRID 47128130. Unpublished.

SPONSOR: BASF Agricultural Products Division, Research Triangle Park, NC, USA.

EXECUTIVE SUMMARY:

& 47128129

In a metabolism study (MRID 47128130) BAS 800 H (100% a.i.), [phenyl-U-^{14}C]-BAS 800 H (>95.0% radiochemical purity) was administered to 4 Wistar rats/sex/group via gavage at 4, 20, or 100 mg/kg bw for plasma kinetics and 5 or 100 mg/kg bw for mass balance, tissue distribution and biliary excretion experiments.

After a single oral administration of 100 mg/kg bw of ^{14}C-BAS 800 H, mean total recoveries of radioactivity were >90% in both sexes. In exhaled air, no administered radioactivity (AR) was excreted as CO_2. After 168 h, the total amounts of radioactivity excreted in urine were 52.6 and 86.6% in males and females, respectively. In feces, 43.3 and 9.8% of AR were recovered in males and females, respectively. The time course of the amount of radioactivity found in urine and feces indicated rapid excretion.

With repeat dosing (14 oral applications with unlabelled BAS 800 H at 100 mg/kg bw and one oral application with labelled ^{14}C-BAS 800 H at 100 mg/kg bw), a similar pattern of excretion was also seen. After 168 h, the total amounts of radioactivity excreted in urine were 67.8 and

83.4% in males and females, respectively. In feces, 35.8 and 13.4% of AR were recovered in males and females, respectively.

After single oral administration of 5 mg/kg bw, mean total recoveries of radioactivity were >90% in males and females. No AR was excreted as CO_2 in exhaled air. Within 168 h 26.0 and 96.1% of AR were excreted in urine of male and female rats, respectively. In feces, 81.2 and 12.8% AR were recovered in males and females, respectively. The time course of the amount of radioactivity found in urine and feces indicated rapid excretion. The results demonstrated a sex-specific excretion pattern for ^{14}C-BAS 800 H with a higher amount of urinary excretion for females than for males. This sex difference was more pronounced at the low dose level than at the high dose level.

A comparable time course of radioactivity was found for blood and plasma in both sexes. A relatively constant blood/plasma ratio was generally found with the observation period of 168 h, indicating that majority radioactivity was in plasma and not bound to cellular blood constituents.

The AUC values for doses of ^{14}C-800 H of 4, 20, and 100 mg/kg bw were 741, 2131, 4502 [µg Eq × h/g] for males and 247, 754, and 3057 [µg Eq × h/g] for females. The findings indicated a sex difference with up to three fold higher internal exposures for males than for females. Increasing the dose by a factor of 25 resulted in an increase of the AUC-values by a factor of 6.1 in males and 12.4 in females. These data indicated a sub-linear correlation of the internal exposure with increasing internal dose.

After a single oral dose of ^{14}C-BAS 800 H at 5 or 100 mg/kg bw, analyses of radioactivity in tissues indicated higher levels in males than in females at respective time points and dose levels, whereas the pattern of distribution in the various organs and tissues was similar in both sexes. Tissue radioactivity concentrations generally declined with time parallel to plasma concentrations. Throughout the time course of the experiments, highest radioactivity levels were generally found in the GI tract, liver, kidneys, lungs, and thyroid whereas radioactivity levels were lowest in brain and bone. Similar findings were noted with repeat dosing.

Within 48 h of administration of ^{14}C-BAS 800 H, excretion of radioactivity in the bile in males and females was 52.3 and 18.9% and 67.8 and 35.5% AR at 5 and 100 mg/kg bw dose levels, respectively. The data indicated higher biliary excretion in males when compared to females.

In conclusion, the biokinetics data demonstrated that orally administered BAS 800 H was rapidly absorbed, distributed, and excreted. Regardless of the dose administered, maximum concentration of BAS 800 H in blood and plasma was reached within 1 h of dosing and declined rapidly after 24 h. The blood and plasma data demonstrated that the majority of the BAS 800 H residues occurred in the plasma and was not bound to cellular elements of the blood. There was a sex dependent difference in the excretion of orally administered BAS 800 H. Following single low- and high-dose administration or a repeat high-dose administration, the main route of elimination in male rats was via the feces, while urinary excretion was the major route of elimination in females. The sex dependent excretion was more pronounced at the low dose level than at the high dose level. The sex dependent difference in excretion of orally dosed BAS 800 H was also demonstrated by the biliary excretion data which showed significantly higher biliary excretion of BAS 800 H residues in males than in females. Excretion of orally dosed BAS 800 H

was essentially complete within 96 h, the majority was eliminated within the first 24 to 48 h. Exhalation was not a relevant excretion pathway of BAS 800 H. At 168 h after dosing, BAS 800 H residues remaining in tissues were very low, and occurred mainly in carcass, liver, skin, and gut contents.

Studies of the metabolic pattern and identification on metabolites in the rats indicated that BAS 800 H was metabolized by three major transformation steps, which were demethylation of the uracil ring system, degradation of the N-methyl-N-isopropyl group to NH2, and cleavage of the uracil ring, forming a sulfonylamide group. The predominant metabolites were M800H01, M800H03, M800H07 and the parent compound. Other minor metabolites were M800H05, M800H16, M800H17, M800H18, M800M19, and M800M20. There were no significant gender differences in metabolic profiles.

This metabolism study in the rat is classified acceptable, guideline and satisfies the guideline requirement for a metabolism study [OPPTS 870.7485, OECD 417] in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

Reviewer #: Steve Wong, Ph.D., Date: August 16, 2008

APPLICANT: BASF Corporation

STUDY TYPE: Metabolism - rat; OPPTS 870.7485; OECD 417.

TEST MATERIAL (PURITY): ^{14}C-BAS 800 H (chemical purity 100%, radiochemical purity 99.9%)

SYNONYMS: N'-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)-pyrimidinyl)benzoyl]-N-isopropyl-N-methylsulfamide-[phenyl-U-^{C}14]; BASF Reg. No. 4054449

CITATION:

Toxicokinetics: PMRA 1546951
Fabian, E. and Landsiedel, R. (2007) ^{14}C-BAS 800 H – Study on the Biokinetics in Rats. Experimental Toxicology and Ecology, BASF AG, Ludwigshafen, Germany, Project No. 02B0627/046008, 24 September 2007. BASF DocID 2007/1033929. Unpublished

Metabolism: PMRA 1546953
Grosshans, F. (2007) The Metabolism of ^{14}C-BAS 800 H (Reg.No, 4054449) in Rats. BASF Agricultural Center Limburgerhof, Germany, Study Code 132617, 17 October 2007. BASF DocID 2006/1035461. Unpublished

SPONSOR: BASF Agricultural Products Division, Research Triangle Park, NC, USA.

EXECUTIVE SUMMARY:

In a toxicokinetic study, [phenyl-U-^{14}C]-BAS 800 H (>95.0% radiochemical purity), was administered to 4 Wistar rats/sex/group via gavage at 4, 20, or 100 mg/kg bw for plasma kinetics and 5 or 100 mg/kg bw for mass balance, tissue distribution and biliary excretion experiments.

After a single oral administration of 100 mg/kg bw of ^{14}C-BAS 800 H, mean total recoveries of radioactivity were >90% in both sexes. In exhaled air, no administered radioactivity (AR) was excreted as CO_2. After 168 h, the total amounts of radioactivity excreted in urine were 52.6 and 86.6% in males and females, respectively. In feces, 43.3 and 9.8% of AR were recovered in males and females, respectively. The time course of the amount of radioactivity found in urine and feces indicated rapid excretion.

With repeat dosing (14 oral doses of unlabelled BAS 800 H at 100 mg/kg bw and one oral application with labelled ^{14}C-BAS 800 H at 100 mg/kg bw), a similar pattern of excretion was also seen. After 168 h, the total amounts of radioactivity excreted in urine were 67.8 and 83.4% in males and females, respectively. In feces, 35.8 and 13.4% of AR were recovered in males and females, respectively.

After single oral administration of 5 mg/kg bw, mean total recoveries of radioactivity were >90% in males and females. No AR was excreted as CO_2 in exhaled air. Within 168 h 26.0 and 96.1% of AR were excreted in urine of male and female rats, respectively. In feces, 81.2 and 12.8% AR were recovered in males and females, respectively. The time course of the amount of radioactivity found in urine and feces indicated rapid excretion. The results demonstrated a sex-specific excretion pattern for ^{14}C-BAS 800 H with a higher amount of urinary excretion for females than for males. This sex difference was more pronounced at the low dose level than at the high dose level.

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A comparable time course of radioactivity was found for blood and plasma in both sexes. A relatively constant blood/plasma ratio was generally found with the observation period of 168 h, indicating that majority radioactivity was in plasma and not bound to cellular blood constituents.

The AUC values for doses of ^{14}C-800 H of 4, 20, and 100 mg/kg bw were 741, 2131, 4502 [µg Eq × h/g] for males and 247, 754, and 3057 [µg Eq × h/g] for females. The findings indicated a sex difference with up to three fold higher internal exposures for males than for females. Increasing the dose by a factor of 25 resulted in an increase of the AUC-values by a factor of 6.1 in males and 12.4 in females. These data indicated a sub-linear correlation of the internal exposure with increasing internal dose.

After a single oral dose of ^{14}C-BAS 800 H at 5 or 100 mg/kg bw, analyses of radioactivity in tissues indicated higher levels in males than in females at respective time points and dose levels, whereas the pattern of distribution in the various organs and tissues was similar in both sexes. Tissue radioactivity concentrations generally declined with time parallel to plasma concentrations. Throughout the time course of the experiments, highest radioactivity levels were generally found in the GI tract, liver, kidneys, lungs, and thyroid whereas radioactivity levels were lowest in brain and bone. Similar findings were noted with repeat dosing.

Within 48 h of administration of ^{14}C-BAS 800 H, excretion of radioactivity in the bile in males and females was 52.3 and 18.9% and 67.8 and 35.5% AR at 5 and 100 mg/kg bw dose levels, respectively. The data indicated higher biliary excretion in males when compared to females.

In conclusion, the biokinetics data demonstrated that orally administered BAS 800 H was rapidly absorbed, distributed, and excreted. Regardless of the dose administered, maximum concentration of BAS 800 H in blood and plasma was reached within 1 h of dosing and declined rapidly after 24 h. The blood and plasma data demonstrated that the majority of the BAS 800 H residues occurred in the plasma and was not bound to cellular elements of the blood. There was a sex dependent difference in the excretion of orally administered BAS 800 H. Following single low- and high-dose administration or a repeat high-dose administration, the main route of elimination in female rats was via the urine, while fecal excretion played a significant role in males. The sex dependent excretion was more pronounced at the low dose level than at the high dose level. The sex dependent difference in excretion of orally dosed BAS 800 H was also demonstrated by the biliary excretion data which showed significantly higher biliary excretion of BAS 800 H residues in males than in females. Excretion of orally dosed BAS 800 H was essentially complete within 96 h, the majority was eliminated within the first 24 to 48 h. Exhalation was not a relevant excretion pathway of BAS 800 H. At 168 h after dosing, BAS 800 H residues remaining in tissues were very low, and occurred mainly in carcass, liver, skin, and gut contents.

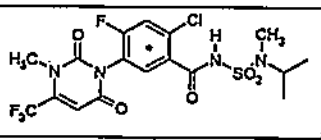
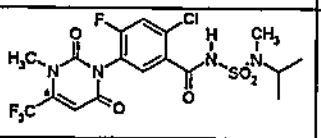
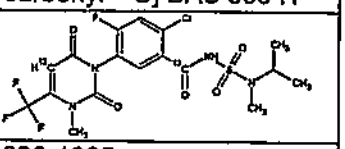
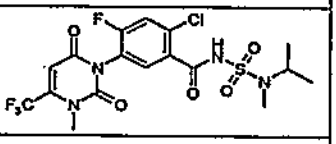
Studies of the metabolic pattern and identification on metabolites in the rats indicated that BAS 800 H was metabolized by three major transformation steps, which were demethylation of the uracil ring system, degradation of the N-methyl-N-isopropyl group to NH_2, and cleavage of the uracil ring, forming a sulfonylamide group. The predominant metabolites were M800H01, M800H03, M800H07 and the parent compound. Other minor metabolites were M800H05, M800H16, M800H17, M800H18, M800M19, and M800M20. There were no significant gender differences in metabolic profiles.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1.

Radiolabelled test material:	[Phenyl-U-^{14}C] BAS 800 H		[Uracil-4-^{14}C] BAS 800 H	[Uracil-5-^{13}C, benzamide-carbonyl-^{13}C] BAS 800 H
Structural formula:				
Lot/Batch #:	825-1085	825-1201	829-1017	828-1085
Radiochemical purity (HPLC):	99.9%	96.0%	99.5%	99.0%
Chemical purity:	100.0%	92.5%	99.2%	
Specific activity: MBq/mg (mCi/mg)	5.54 (0.15)	5.19	4.26 (0.12)	
Non-radiolabelled test material:		BAS 800 H		
Lot/Batch #:		L67-140	COD-000298	
Chemical purity:		99.9%	93.9%	

2. **Vehicle and/or positive control:** 0.5% carboxymethylcellulose in double distilled water.

3. **Test animals:**

Species:	Rats	
Strain:	Crl:WI (Han)	
Age/weight at study initiation:	Age: ≥8 weeks; Mean weight: ♂ = ~290; ♀ = ~210 g	
Source:	Charles River, Sulzfeld, Germany	
Housing:	Individually in cages; during acclimatization and prior to the experiment in type III Macrolon cages; during balance experiments in all-glass metabolism cages; type Metabowl (Jencons, Leighton Buzzard, UK); in restriction cages (BASF AG, Ludwigshafen, FRG) for experiments on biliary/excretion and in steel wire mesh cages (Becker & Co., Castrop-Rauxel, FRG) for tissue distribution experiments and blood/plasma kinetics.	
Diet:	Kliba lab diet mouse/rat "GLP", pellet (during acclimation) or meal (when in metabolism cages), supplied by Provimi Kliba SA, Kaiseraugst, Switzerland, *ad libitum*	
Water:	Tap water *ad libitum*	
Environmental conditions:	Temperature:	20-24°C
	Humidity:	30-70%
	Air changes:	no information
	Photoperiod:	natural day/night rhythm with additional artificial light as required during working hours.
Acclimation period:	at least 3 days prior to administration	

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B. STUDY DESIGN:

1. Experimental dates: Toxicokinetic study: Start: November 19, 2004 End: January 31, 2007
Metabolism study: Start: October 26, 2004 End: May 31, 2006

2. Animal assignment: Animals were assigned to test groups via a randomization protocol provided by a computer. The test groups are noted in Table 1a and 1b.

Table 1a: Study design (Pharmacokinetics)

	Pharmacokinetic			Mass balance, tissue distribution, bile excretion			Tissue distribution		Excretion via bile	
Test material	phenyl-U-^{14}C-BAS 800 H			phenyl-U-^{14}C-BAS 800 H			phenyl-U-^{14}C-BAS 800 H		^{13}C & phenyl-U-^{14}C-BAS 800 H	
mg/kg bw	4	20	100	5	100	100 (14 daily doses + 1 radio-active dose on day 15)	5	100	5	100
N/sex	4	4	4	4	4	4	12	12	4	4
Assess-ment	radioactivity in whole blood and plasma; assessed at 1, 2, 4, 8, 24, 48, 72, 96, 120, 144, 168 h post-dosing			Urine collected at 6, 12, 24, 48, 72, 96, 120, 144, 168 h; Feces collected at 24, ... 168 h; Tissues at sacrifice at 168 h; Cage washing			3/sex sacrificed at 1, 4 (♀), 20 (♀), 24, 48 (♂), 72 (♂); tissue radioactivity	3/sex sacrificed at 1, 7, 20, 34 h; tissue radio-activity	Bile duct cannulated, bile collected at 3 h intervals up to 48 h; radioactivity in bile	
				Expired air collected for 48 h (only 2♂/dose)						
metabolites	no			yes		yes	no		yes	
sacrifice	168 h			168 h			1, 7, 20, 34 h		48 h	

Table 1b: Study design (Metabolism)

Test material	phenyl-U-^{14}C-BAS 800 H			uracil-4-^{14}C-BAS 800 H		
mg/kg bw	5	100	100	5	100	100
N/sex	4	4	10	4	4	10
sacrifice	168 h		168 h	168 h		168 h
Metabolites in	liver, kidneys, plasma, fat		urine, feces	liver, kidneys, plasma, fat		urine, feces

3. Test substance preparation and analysis:

The stability, homogeneity and correctness of the concentration of the test item preparations as well as the radiochemical purity of ^{14}C-BAS 800 H were checked via HPLC.

To facilitate the structure elucidation of formed metabolites, ^{13}C-BAS 800 H was added to the BAS 800 H preparation for the bile excretion experiment at the high dose level. In general ^{14}C-BAS 800 H and the non-radiolabelled BAS 800 H (^{12}C- and ^{13}C-BAS 800 H) were prepared in 0.5 % carboxymethylcellulose in double distilled water under the addition of a respective amount of Cremophor. About 10 mL/kg bw of a preparation was administered to rats by gavage.

In order to achieve the required specific activity, appropriate amounts of non-radiolabelled BAS 800 H were added to the respective amounts of a stock solution of radiolabelled BAS 800 H and the aqueous vehicle (0.5 % carboxymethylcellulose in double distilled water) was added and filled up to the final volume. The activity per animal was about 0.5-2 MBq.

For the bile excretion experiment at the high dose, ^{13}C-labelled Bas 800 H was mixed with unlabelled BAS 800 H 1:2 (w:w) and in order to achieve the required specific activity, this mixture was added to the respective amounts of a stock solution of radiolabelled material and the aqueous vehicle was added and filled up to the final volume. The quantity of radioactivity per animal was about 0.5-2 MBq. The stability, homogeneity, and concentrations of BAS 800 H in the aqueous vehicle were verified by HPLC.

Results

No analytical data on the analyses of stability, homogeneity, and concentration were given in the Report, which simply stated that

"*The analytical investigations performed in the context of this study demonstrated the stability, homogeneity and correctness of the concentrations of C-BAS 800 H in the vehicle.*"

Statistics:

Means were calculated for individual parameters. Analysis of kinetic data was performed based on the group mean values using the PC program system TOPFIT Version 2.0 (1).

C. METHODS:

1. Administration of BAS 800 H preparations:

The BAS 800 H preparation was administered by oral gavage in a volume of 1 mL/100 g bw.

a. Radioactivity in whole blood and plasma:

Rats (4/sex/group) were administered radioactive BAS 800 H preparations at 4, 20, or 100 mg/kg bw. After dosing, blood samples (~100-200 µL) were taken from the retro-orbital sinus or by exsanguinations at the last time point at 1, 2, 4, 8, 24, 48, 72, 96, 120, 144, and 168 h. Whole blood and plasma samples were checked for total radioactivity.

b. Balance/excretion:

Rats (4/sex/group) were given a single oral dose of radioactive BAS 800 H preparations at 5, or 100 mg/kg bw. A group of rats, 4/sex, was given non-radioactive BAS 800 H daily for 14 days at 100 mg/kg bw/d and followed with a radioactive BAS 800 H preparation at 100 mg/kg bw on day 15. After dosing, the rats were placed in individual metabolism cages. Urine was collected at 6, 12, 24, 48, 72, 96, 120, 144, and 168 h. Feces was collected at 24, 48, 72, 96, 120, 144, and 168 h. Expired air was collected for 48 h from the first 2 males that received a single dose of radioactive BAS 800 H at 5 and 100 mg/kg bw. The collected urine, feces, and expired air samples were analyzed for radioactivity. At 168 h post-dosing, the rats were sacrificed and the following tissues were collected, processed, and analyzed for radioactivity.

Tissues collected for radioactivity analysis:
Heart, liver, spleen, bone, skin, lungs, ovaries, muscle, kidneys, testes, brain, pancreas, uterus, adipose tissue, stomach and stomach contents, thyroid, adrenal, bone marrow, blood cells and plasma, and carcass.

c. Tissue distribution:

Rats (12/sex/group) were given single oral dose of radioactive BAS 800 H at 5 or 100 mg/kg bw. Following dosing, 3 rats/sex/group were sacrificed at the time intervals given below. The time points selected corresponded to the time points of maximum plasma concentration (MPC), ½ MPC, ¼ MPC and 1/8 MPC which were determined based on the results of earlier experiments. Tissues from the sacrificed rats were collected and analyzed for radioactivity.

Sacrifice time intervals:
5 mg/kg bw group: 1, 4 (♀), 20 (♀), 24, 48 (♂), and 72 (♂) h post-dosing
100 mg/kg bw group: 1, 7, 20, 34 h post-dosing

Tissues collected for analysis of radioactivity:
Heart, liver, spleen, bone, skin, lungs, ovaries, muscle, kidneys, testes, brain, pancreas, uterus, adipose tissue, stomach and stomach contents, thyroid, adrenal, bone marrow, blood cells and plasma, and carcass.

d. Bile excretion of radioactivity:

In this experiment, the bile duct of rats were cannulated and the rats (4/sex/group) were given a single oral dose of radioactive BAS 800 H at 5 or 100 mg/kg bw. Bile was collected at 3 h intervals for up to 48 h and analyzed for radioactivity.

e. Metabolism study (metabolic pattern and isolation and identification of urinary and fecal metabolites):

For the isolation and identification of urinary and fecal metabolites, groups of male and female rats were administered uracil-label14-BAS 800 H or phenyl-label14C-BAS 800 H at 100 mg/kg bw.

Bile fluid collected from bile-cannulated male and female rats in the biokinetics study (at single oral doses of 5 or 100 mg/kg bw) was used for identification of metabolites in bile fluid.

For the analysis of the metabolite patterns in liver, kidney, fat, and plasma, male and female rats were dosed at 5 or 100 mg/kg bw (uracil- and phenyl-label), the organs were removed 1 h after dosing.

The biomaterials were processed and analyzed using appropriate methods (combustion of solid samples, extraction, isolation, and purification, liquid scintillation counting, high performance liquid chromatography, mass spectrometry, NMR spectrometry, and co-chromatography)

2. Analysis of radioactivity:

All samples of biological materials were prepared for radioactivity analysis using conventional methods. For tissues, blood cells, plasma, adipose tissue, bone, muscle the calculation of % of administered dose (AD) was based on the sample amount taken for the measurement. For all other tissues, the calculation was based on the weight of the whole tissue.

II. RESULTS

A. Plasma and blood levels of ^{14}C-BAS 800 H: Table 2

Orally administered ^{14}C-BAS 800 H was rapidly absorbed and the maximum plasma concentration (C_{max}) was reached within 1 h of dosing (Time to reach maximum concentration, T_{max}) for all dose groups. Thereafter the plasma level of ^{14}C-BAS 800 H declined rapidly and only residual radioactivity was detected at 168 h. A comparable time course of radioactivity was found in blood and plasma of both sexes. A relatively constant blood/plasma ratio between 0.1 and 0.4 was generally found, indicating that major parts of the radioactivity were in plasma and not bound to cellular blood constituents.

The area under the plasma concentration time curve (AUC) values indicated a gender difference with up to three fold higher internal exposures for males than for females, most probably due to a higher clearance of ^{14}C-BAS 800 H in female rats. Increasing the dose by a factor of 25 resulted in an increase of the AUC-values by a factor of 6.1 in males and 2.4 in females. These data indicated a sub-linear correlation of the internal exposure with increasing external dose.

Table 2: Mean plasma and blood levels of radioactivity after single oral administration of phenyl-U-^{14}C-BAS 800 H.

		♂			♀		
mg/kg bw		4	20	100	4	20	100
N		4	4	4	4	4	4
Mean plasma concentration of radioactivity, µg Eq/g plasma	1 h	23.9±4.1	98.3±19.3	286.0±27.5	23.0±8.9	84.8±2.9	258.3±88.5
	2 h	22.5±3.4	84.3±15.9	255.2±22.4	16.7±7.1	66.5±8.4	219.2±73.1
	4 h	21.2±3.2	72.6±27.0	220.5±26.0	12.4±6.2	44.3±10.5	138.4±52.5
	8 h	19.4±3.4	55.0±32.7	145.9±34.4	9.6±5.2	30.1±9.8	96.7±48.2
	24 h	11.4±4.3	29.6±27.4	48.7±30.7	2.3±1.6	6.4±3.2	38.0±29.3
	48 h	5.1±3.1	13.6±17.1	15.8±16.9	0.4±0.3	1.1±0.7	6.1±4.4
	72 h	2.4±2.2	6.5±9.3	7.0±9.3	0.1±0.1	0.4±0.2	2.1±1.6
	96 h	0.9±1.1	2.7±3.9	3.7±5.0	0.1±0.1	0.5±0.3	2.0±1.5
	120 h	0.4±0.6	1.4±1.9	2.3±3.0	0.1±0.1	0.3±0.2	1.5±0.9
	144 h	0.2±0.3	0.7±0.9	1.4±1.9	0.1±0.1	0.3±0.2	1.0±0.7
	168 h	0.1±0.1	0.4±0.4	1.0±1.1	0±0.1	0.2±0.1	0.7±0.4
Mean blood concentration of radioactivity, µg Eq/g plasma	1 h	3.9±1.6	21.2±10.1	104.5±38.5	3.3±1.5	17.3±7.3	51.9±25.7
	2 h	3.6±1.6	20.7±5.9	73.8±13.1	1.8±0.8	10.4±3.4	58.1±29.4
	4 h	2.5±1.0	7.5±2.3	72.9±13.1	1.2±1.0	7.6±5.3	22.3±9.0
	8 h	1.5±0.2	4.9±4.6	34.5±14.3	1.3±0.7	3.0±1.7	21.7±18.0
	24 h	2.3±2.4	9.4±10.7	9.9±6.5	0.6±0.6	1.0±0.5	9.7±7.8
	48 h	1.4±1.0	3.4±3.8	4.1±4.9	0.1±0.1	0.3±0.2	1.5±0.8
	72 h	0.2±0.3	0.7±0.7	0.9±1.1	0.0±0.0	0.1±0.1	0.4±0.2
	96 h	0.2±0.3	1.0±1.7	1.8±2.7	0.0±0.0	0.1±0.1	0.4±0.2
	120 h	0.1±0.1	0.2±0.2	0.3±0.3	0.0±0.0	0.1±0.1	0.3±0.1
	144 h	0.0±0.0	0.1±0.1	0.4±0.4	0.0±0.0	0.1±0.0	0.4±0.3
	168 h	0.0±0.0	0.1±0.1	0.4±0.4	0.0±0.0	0.0±0.0	0.3±0.2
Blood/plasma concentration ratio	1 h	0.164	0.216	0.365	0.143	0.204	0.201
	2 h	0.159	0.245	0.289	0.107	0.156	0.265
	4 h	0.118	0.103	0.331	0.097	0.172	0.161
	8 h	0.076	0.089	0.237	0.137	0.100	0.225
	24 h	0.198	0.318	0.203	0.258	0.149	0.255
	48 h	0.283	0.252	0.256	0.250	0.290	0.241
	72 h	0.100	0.100	0.131	0.167	0.205	0.203
	96 h	0.234	0.349	0.468	0.333	0.235	0.189
	120 h	0.167	0.164	0.145	0.286	0.375	0.200
	144 h	0.095	0.197	0.287	0.200	0.280	0.379
	168 h	0.200	0.194	0.442	0.333	0.267	0.408
C_{max}	µg Eq/g	23.9	98.3	286.0	23.0	84.8	258.3
T_{max}	h post-dosing	1	1	1	1	1	1
Initial half life	h	20.9	22.6	33.5	49.5	58.1	59.2
AUC	µg Eq*h/g	741	2131	4502	247	754	3057

Data taken from Tables 2, 14-20; pages 38, 49-55 of Biokinetics Report.
C_{max} = maximum plasma concentration; T_{max} = time to reach maximum plasma concentration; AUC = calculated area under the plasma concentration time curve

B. Balance and excretion pattern of ^{14}C-BAS 800 H:

After single or repeat oral administration of ^{14}C-BAS 800 H, mean total recoveries of radioactivity in the urine and feces were high for all groups (97-110% AD at 168 h). Radioactivity in expired air was low and exhalation was not a relevant excretion pathway of BAS 800 H. In general, the majority of radioactivity was excreted in the urine and feces within the first 24 to 48 h and excretion was essentially complete within 96 h. Radioactivity remaining in tissues at 168 h was very low, and occurred mainly in the carcass (0.06-0.10%), liver (0.03-0.06%), skin (0.02-0.05%), and gut content (0.02%).

The data demonstrated a sex specific excretion pattern for ^{14}C-BAS 800 H with a higher amount of

urinary excretion for females than for males. The sex dependent excretion was more pronounced at the low dose level than at the high dose level.

Table 3: Mean (% administered dose, AD) excretion and tissue retention of radioactivity after oral administration of phenyl-^{14}C-BAS 800 H.

		♂			♀		
mg/kg bw		5	100	100 (14+1)	5	100	100 (14+1)
N		4	4	4	4	4	4
Mean urine radioactivity	0-6 h	6.11±3.31	28.10±12.44	39.78±4.26	30.96±20.07	57.08±6.49	55.99±4.77
	6-12 h	3.61±2.94	9.53±2.89	10.03±4.73	15.38±7.77	8.72±1.90	6.23±1.01
	12-24 h	6.50±5.46	10.26±3.97	8.73±1.09	29.55±11.02	14.11±4.21	10.78±1.33
	24-48 h	5.37±3.97	3.26±1.44	1.91±0.52	14.06±6.90	3.38±1.59	5.25±1.65
	48-72 h	2.40±2.55	0.79±0.25	0.63±0.22	3.56±2.23	1.25±0.64	2.09±0.79
	72-96 h	1.20±1.61	0.34±0.17	0.22±0.05	1.11±0.42	0.71±0.43	1.11±0.40
	96-120 h	0.36±0.28	0.16±0.07	0.13±0.04	0.56±0.31	0.54±0.32	0.63±0.06
	120-144 h	0.17±0.13	0.10±0.05	0.08±0.03	0.45±0.39	0.42±0.09	0.67±0.16
	144-168 h	0.11±0.07	0.06±0.03	0.29±0.45	0.43±0.28	0.36±0.10	0.63±0.33
	Urine total	26.02±16.06	52.61±10.11	61.80±3.63	96.06±7.88	86.56±2.68	83.38±2.43
Mean fecal radioactivity	0-24 h	30.6±21.98	21.14±6.66	24.31±4.89	6.55±1.46	7.93±2.48	9.64±3.06
	24-48 h	30.05±9.91	18.24±7.43	9.84±2.83	4.71±3.37	1.34±0.47	2.80±0.98
	48-72 h	6.95±3.17	2.92±0.71	1.02±0.19	1.11±0.68	0.24±0.18	0.52±0.24
	72-96 h	11.21±20.04	0.58±0.34	0.30±0.14	0.21±0.15	0.10±0.05	0.14±0.08
	96-120 h	0.72±0.96	0.20±0.12	0.12±0.06	0.09±0.05	0.07±0.03	0.09±0.03
	120-144 h	1.61±2.93	0.18±0.08	0.07±0.03	0.09±0.07	0.05±0.01	0.10±0.06
	144-168 h	0.07±0.02	0.09±0.04	0.10±0.07	0.04±0.03	0.09±0.09	0.09±0.03
	Fecal total	81.20±16.41	43.33±8.11	35.76±3.80	12.80±4.38	9.82±2.22	13.38±2.44
Cage wash	168 h	0.56±0.59	0.69±0.31	0.58±0.28	1.34±0.65	0.80±0.25	1.69±0.45
Carcass	168 h	0.06±0.01	0.07±0.03	0.05±0.01	0.10±0.05	0.08±0.04	0.05±0.02
Skin	168 h	0.02±0.0	0.03±0.02	0.02±0.01	0.03±0.01	0.02±0.01	0.03±0.01
Liver	168 h	0.03±0.01	0.02±0.01	0.02±0.01	0.06±0.02	0.03±0.02	0.05±0.02
Gut content	168 h	0.02±0.0	0.03±0.02	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01
Kidneys	168 h	0.01±0.01	0.0	0.0	0.01±0	0.0	0.0
Total	168 h	107.90±3.59	96.77±3.31	98.24±0.15	110.41±3.18	97.32±1.18	98.60±0.09
Data taken from Tables 1, 8-13; pages 37, 43-48 of Biokinetics Report.							

Table 4: Comparison of time course of mean excretion of radioactivity (% administered dose, AD) after a single oral administration of phenyl- or uracil-labelled ^{14}C-BAS 800 H at 100 mg/kg bw.

	% metabolites excreted in urine and feces (metabolism study)							
	100 mg phenyl^{14}C-BAS 800 H/kg bw				100 mg uracil^{14}C-BAS 800 H/kg bw			
	urine		feces		urine		feces	
	♂	♀	♂	♀	♂	♀	♂	♀
0-24 h	26.0	44.3	32.1	9.68	26.5	48.0	29.1	10.4
24-48 h	1.55	3.06	11.8	3.47	1.85	2.42	15.7	1.02
48-72 h	0.43	1.23	2.51	0.93	0.45	1.13	3.79	0.65
72-96 h	0.47	0.89	0.66	0.45	0.11	0.59	1.16	0.27
96-120 h	-	-	-	-	-	0.28	-	0.11
120-144 h	-	-	-	-	-	0.29	-	0.15
144-168 h	-	-	-	-	-	0.85	-	0.14
0-72 h	-	-	-	-	-	-	-	-
0-96 h	28.5	49.5	47.0	14.5	28.9	-	49.8	-
0-168 h	-	-				53.5	-	13.7
Data taken from Tables 6, page 61 of Metabolism Report.								

C. Tissue distribution of ^{14}C-BAS 800 H: Tables 5-7

Within 1 h after oral administration of ^{14}C-BAS 800 H at 5 or 100 mg/kg bw, the highest radioactivity was found in the stomach and gut contents, carcass, liver, and skin. Thereafter, radioactivity in the gut

content increased as the ingested ^{14}C-BAS 800 H passed down the GI tract. In general, radioactive residues in tissues were rather low. Radioactivity generally declined continuously in organs and tissues of both sexes during the following hours in parallel to the concentration in plasma. After 20-24 h, only negligible radioactivity was measured in internal organs except for the liver. By 168 h, all internal tissues in male and female rats that received a single oral dose of ^{14}C-BAS 800 H at 5 or 100 mg/kg bw contained very low radioactivity (<0.1 and <1.0 µg Eq/g at 5 and 100 mg/kg bw, respectively). Similar findings of low radioactivity in internal tissues (<0.5 0 µg Eq/g except for the liver of females) were also observed in male and female rats that were given 15 daily oral doses of BAS 800 H (14 X BAS 800 H; 1 X ^{14}C-BAS 800 H on day 15) at 100 mg/kg bw and sacrificed 168 h later.

Table 5: Mean (% administered dose; mean±SD) radioactivity in tissues after oral administration of ^{14}C-BAS 800 H at 5 and 100 mg/kg bw (Only tissues with ≥0.10% AD are listed).

	♂				♀			
	5 mg/kg bw (N = 3/sacrifice interval)							
Time, h	1	24	48	72	1	4	20	24
liver	22.9±0.82	7.74±0.74	2.29±2.46	0.57±0.43	25.9±5.71	19.6±3.52	4.20±1.39	4.18±1.54
carcass	20.4±1.71	8.67±5.04	2.29±2.21	0.71±0.50	14.8±4.53	7.98±3.21	3.03±0.67	2.02±1.83
stomach content	14.1±5.82				12.6±0.73	3.62±1.67		
skin	8.88±1.41	3.42±2.54	0.92±1.00	0.30±0.21	5.57±1.26	3.42±1.88	1.29±0.45	1.13±0.71
gut content	7.21±0.29	19.9±3.03	3.88±1.42	1.52±1.03	5.47±0.46	8.60±1.09	2.58±1.50	3.86±2.18
gut	3.29±0.13	2.73±0.47	0.49±0.27	0.28±0.15	2.86±0.23	1.69±0.26	0.48±0.20	0.53±0.33
plasma	3.11±0.19	1.05±0.91	0.32±0.47		2.56±1.68	1.04±0.68	0.35±0.12	0.21±0.23
stomach	1.84±0.27				1.94±0.55	1.48±0.70		
blood cells	2.03±0.43	0.46±0.29			1.51±0.67	0.63±0.25	0.24±0.02	0.12±0.15
kidneys	1.00±0.04	0.27±0.11			1.14±0.20	0.61±0.04	0.12±0.03	0.10±0.05
lungs	0.80±0.19	0.28±0.18			0.53±0.18	0.40±0.21	0.11±0.04	
heart	0.34±0.01				0.33±0.27	0.11±0.06		
testes/ovaries	0.24±0.05				0.21±0.08	0.15±0.12		
uterus					0.25±0.20	0.13±0.09		
	100 mg/kg bw (N = 3/sacrifice interval)							
Time, h	1	7	20	34	1	7	20	34
stomach content	24.1±14.0	6.13±2.64			29.4±4.30	3.69±3.15		
carcass	16.3±6.17	7.28±1.71	2.99±1.78	1.51±0.11	7.33±0.64	2.70±0.26	1.09±0.38	0.96±0.22
gut content	13.9±2.26	24.5±1.22	14.2±7.59	6.08±3.35	18.8±3.14	13.2±3.49	3.90±1.46	1.81±1.24
liver	8.95±2.18	4.79±1.38	1.80±0.26	1.41±0.16	6.68±0.38	3.32±0.16	1.39±0.39	1.16±0.11
skin	8.71±3.19	3.69±1.58	1.44±0.67	0.90±0.16	5.21±0.67	1.92±0.37	0.92±0.11	0.67±0.15
gut	4.14±1.22	2.02±0.77	0.79±0.43	0.24±0.10	4.28±1.01	1.06±0.23	0.36±0.06	0.14±0.05
stomach	2.23±0.48	0.75±0.25			2.67±1.39	0.87±1.06		
plasma	1.90±0.46	0.88±0.59	0.43±0.34	0.29±0.10	1.95±0.4	0.69±0.10	0.22±0.17	0.17±0.09
lungs	1.62±2.25	0.16±0.06			0.30±0.07	0.14±0.06		
kidneys	1.50±0.21	0.59±0.24	0.10±0.05		1.02±0.05	0.25±0.02		
blood cells	1.07±0.40	0.38±0.12	0.11±0.08		0.70±0.07	0.20±0.06	0.12±0.10	
heart	0.34±0.16	0.15±0.13			0.13±0.03			
testes/ovaries	0.27±0.09	0.21±0.02						
muscle	0.11±0.04							
Data taken from Tables 25-28 and 34-37; pages 60-63, 69-72 of Biokinetics Report.								

Table 6: Mean (µg Eq/g tissue; mean±SD; only tissues with ≥1.00 µg Eq/g are listed) radioactivity in tissues after oral administration of ^{14}C-BAS 800 H at 5 mg/kg bw.

	5 mg/kg bw (N = 3/sacrifice interval)							
	♂				♀			
Time, h	1	24	48	72	1	4	20	24
stomach content	66.2±11.8				70.2±22.6	26.3±12.7		
liver	34.0±3.01	11.5±0.69	3.08±3.45		38.3±9.62	27.1±5.98	6.22±1.44	5.76±2.14
plasma	30.4±3.42	9.26±7.39	1.71±1.97		18.7±11.4	8.07±4.76	2.92±0.01	1.74±2.09
stomach	19.8±1.53				18.4±4.91	13.6±6.90		
thyroid	9.72±[illegible].90	4.23±3.20			6.02±3.21	2.05±1.73	1.00±0.09	
blood cells	8.34±2.07	1.90±1.19			5.42±2.49	2.37±1.01		
lungs	8.16±1.25	2.77±1.92			5.07±2.11	2.97±1.41		
gut content	8.01±0.29	21.4±3.36	4.03±2.01	1.65±1.13	6.52±0.50	9.47±1.30	2.82±1.16	3.39±1.49
kidneys	8.00±0.58	2.05±0.78			8.71±1.32	4.39±0.12		
gut	7.94±0.69	6.45±0.88	1.76±1.06		5.70±0.37	3.40±0.52	1.07±0.43	1.10±0.65
adrenals	5.98±1.44	1.51±1.01			3.30±1.62	1.83±1.04		
heart	5.04±0.52	1.50±1.09			4.45±3.48	1.55±0.72		
bone marrow	3.21±0.44				1.93±0.89	1.02±0.63		
skin	2.68±0.35	1.14±0.88			1.86±0.42	1.23±0.68		
pancreas	2.22±0.24				1.66±0.69			
spleen	1.82±0.16				1.26±0.44			
carcass	1.77±0.14				1.34±0.47			
testes/ovaries	1.27±0.22				3.35±1.74	1.75±0.72		
uterus	-	-	-	-	1.14±1.30	2.02±0.66		
muscle	1.25±0.13							
bone	1.01±0.11							
Data taken from Tables 5, 30-33; pages 40, 65-68 of Biokinetics Report.								

Table 7: Mean (µg Eq/g tissue; mean±SD; only tissues with ≥5.00 µg Eq/g are listed) radioactivity in tissues after oral administration of ^{14}C-BAS 800 H at 100 mg/kg bw.

	100 mg/kg bw (N = 3/sacrifice interval)							
	♂				♀			
Time, h	1	7	20	34	1	7	20	34
stomach content	1214±471	577±96.4	22.2±27.7		3514±1279	422±258		8.79±10.8
liver	224±45.3	117±35.6	44.9±7.31	38.1±5.35	188±7.87	86.7±7.39	38.9±9.17	35.6±5.35
plasma	222±40.7	108±43.9	50±35	29.9±7.01	182±14.5	69.3±9.62	24.4±17.4	23.6±9.72
stomach	421.6±121	138±42.4	12.4±10.4		484±184	158±188		
blood cells	74.0±23.0	28.0±10.2	8.30±5.59	5.80±2.15	43.2±2.66	12.9±5.7	7.70±5.94	6.57±2.44
thyroid	79.3±21.6	46.2±17.3	11.6±7.33	8.60±1.53	85.6±20.8	29.2±3.70	11.5±8.55	9.54±3.00
lungs	206±237	30.5±11.8	13.5±9.29	8.33±1.84	60.1±11.2	22.6±5.01	9.21±6.15	8.24±2.51
kidneys	198±19.2	79.3±36.7	13.8±5.83	8.62±0.67	143±8.90	31.4±3.48	9.09±3.10	7.34±0.48
gut	131±27.8	62.0±15.1	28.8±15.8	8.69±3.28	153±29.4	40.0±3.35	14.7±3.63	5.14±1.14
gut content	319±13.9	504±83.0	278±160	160±82.3	417±94.7	299±68.6	78.8±12.4	44.3±13.9
adrenals	81.0±55.0	23.1±8.81	9.20±5.46	5.00±0.43	46.6±8.43	15.7±2.11	5.45±2.88	
heart	79.4±30.2	32.0±20.0	9.39±6.22	6.01±1.78	40.2±6.04	13.6±2.00		
bone marrow	49.8±24.5	21.3±7.21	9.27±5.82		37.7±6.76	14.2±3.61	7.13±5.64	
pancreas	42.1±16.2	13.6±5.55	5.44±3.33		25.8±6.40	8.40±0.82		
spleen	36.4±15.2	10.3±3.33			18.4±2.13	5.28±0.54		
testes/ovaries	26.1±7.78	19.6±3.76	7.51±4.86		41.9±9.00	15.0±3.25	5.85±3.67	
uterus	-	-	-	-	54.6±11.4	22.4±5.42	8.10±5.04	7.04±2.01
skin	55.0±19.9	23.6±9.75	9.68±4.68	5.73±0.92	35.8±6.06	13.4±1.83	6.70±1.65	
carcass	30.7±12.5	13.6±3.28	5,56±3.29		13.2±0.85			
Data taken from Tables 4, 21-24; pages 39, 56-59 of Biokinetics Report.								

D. Excretion of ^{14}C-BAS 800 H via bile: Table 8

Regardless of the dose level administered, there was a sex dependent difference in biliary excretion of ^{14}C-BAS 800 H. Significantly higher percentages of orally administered BAS 800 H were excreted via the bile in males than in females.

Table 8: Mean (mean±SD) percentages of biliary excretion of radioactivity after a single oral administration of ^{14}C-BAS 800 H at 5 or 100 mg/kg bw.

		♂		♀	
mg/kg bw		5	100	5	100
Time interval, h	0-3	2.82±1.91	5.01±3.41	1.10±0.34	2.14±1.16
	3-6	4.59±2.66	8.15±6.47	1.09±0.21	1.78±0.72
	6-9	4.69±2.29	6.59±5.21	0.81±0.21	1.46±0.64
	9-12	5.33±2.24	6.24±3.58	0.77±0.14	1.94±1.18
	12-15	5.99±2.01	6.94±1.02	0.77±0.07	3.41±2.44
	15-18	5.99±1.16	7.60±1.68	0.91±0.15	3.20±1.05
	18-21	4.64±0.93	5.29±1.47	1.24±0.21	3.29±0.88
	21-24	3.32±0.81	4.28±2.69	1.23±0.47	3.18±0.75
	24-27	2.52±0.61	3.36±2.13	0.98±0.71	3.11±0.90
	27-30	2.21±0.53	2.64±1.63	0.88±0.88	2.70±1.21
	30-33	2.01±0.39	2.26±1.36	1.03±0.55	2.35±1.14
	33-36	1.76±0.27	2.41±2.08	1.58±0.45	2.11±0.84
	36-39	1.62±0.55	2.44±2.48	1.63±0.42	1.69±0.59
	39-42	1.63±0.38	1.88±1.82	1.76±0.36	1.33±0.42
	42-45	1.71±0.48	1.47±1.29	1.51±0.24	0.87±0.23
	45-48	1.47±0.55	1.18±1.01	1.13±0.43	0.92±0.15
	Total	52.3±13.4	67.8±6.16	18.4±3.36	35.5±4.94
Data taken from Tables 40-41, pages 75-76 of Biokinetics Report					

E. Metabolic pattern and identification of metabolites: Tables 9-10

Metabolites in urine:

The unchanged parent compound was predominant in all dose groups. BAS 800 H amounted to 10.9-48.2 % of the administered dose (AD) for male rats and to 48.7-88.9 % of the dose female rats.

The major metabolites identified in urine samples of male rats were M800H01 (3.5-9.1% AD), M800H03 (0.8-2.3% AD), M800H05 (0.4-4.2% AD), and M800H07 (0.4-2.2% AD). In urine samples of female rats, the metabolite M800H07 (0.6-4.6% AD) was the predominant one; the other metabolites amounted to 0.05-1.7% AD. M800H09 occurred in small amounts (<0.01-0.3% AD) in urine both males and females.

M800H37 (a phenyl-label specific metabolite), M800H23 (a uracil-label specific metabolite), M800H02 (both phenyl- and uracil-label), M800H06 (both phenyl- and uracil-label) were also detected in small amounts in the urine of male and female rats.

Total identified metabolites in urine (both labels) were in the range of 25-62% of the dose for male rats and 49-95% of the dose for female rats (both labels).

Metabolites in feces:

The predominant metabolites in feces of male and female rats in all dose groups were M800H01 (18.1-43.9% AD in males and 1.0-2.5% AD in females) and the unchanged parent compound (3.8-16.2 and 2.9-9.9% AD in males and females, respectively). Other metabolites identified in feces were M800H02, M800H03 (♂ = 2.1-5.2, ♀ = 0.4-1.6% AD), M800H04, M800H05 (♂ = 0.3-3.6, ♀ <0.01-0.2% AD), M800H06, M800H07 (♂ = 1.1-2.0, ♀ = 0.7-1.4% AD) and M800H08. The HPLC retention behaviour of the metabolites M800H02 and M800H06, as well as of the metabolites M800H04 and M800H08 were very similar; therefore the peaks were not clearly separated by HPLC. The sum of M800H02 and M800H06 amounted to 2.4-8.6% AD for male rats and 0.2-0.6% AD for female rats. The sum of M800H04 and M800H08 amounted to 0.7-2.4% AD for male rats and to 0.6-1.2% AD for female rats. The phenyl-label specific metabolite M800H37 was found in amounts of 0.8-4.5 % and <0.01-0.2 % AD for male and female rats, respectively. Metabolite M800H09 was only detected in minor amounts (0.03%

AD) in feces of female rats. The uracil-label specific metabolite M800H23 was not detected in feces of both genders.

Total identified metabolites in feces were in the range of 32-72% AD for male rats and 8-13% AD for female rats.

Table 9: Identified metabolites (% administered dose) in urine and feces of rats 72-168 h after single oral administration of ^{14}C-BAS 800 H (phenyl-label) at 5 mg/kg bw and after 48-168 h after repeat daily administration of ^{14}C-BAS 800 H (phenyl-label) at 100 mg/kg bw.

	phenyl^{14}C-BAS 800 H/kg bw							
administration	Single 5 mg				14+1 doses; 100 mg/kg bw/d			
bio-sample	urine		feces		urine		feces	
sex	♂	♀	♂	♀	♂	♀	♂	♀
h assessed	0-168		0-96	0-72	0-168		0-72	0-48
BAS 800 H	10.9	88.9	3.77	2.93	48.2	78.1	5.55	5.25
M800H01	5.21	0.85	43.9	2.52	7.88	1.70	18.08	1.63
M800H05	4.23	0.09	3.55	0.14	1.45	-	0.867	0.09
M800H03	2.34	0.67	5.22	1.63	1.96	0.43	2.092	0.84
M800H07	1.65	4.62	2.01	1.39	1.86	2.54	1.136	1.12
M800H37	0.54	0.01	4.50	0.13	0.16	-	1.015	0.20
M800H02+06	0.04	-	8.64	0.58	0.04	-	2.387	0.51
M800H09	0.02	-	-	0.03	0.002	0.02	-	-
M800H04+08	nd	-	0.68	0.97	nd	-	1.177	1.23
total	25.0	95.2	72.3	10.3	61.6	82.7	32.3	10.9

Data taken from Tables 10-13, 18-21, pages 63-65 and 67-69 of Metabolism Report; nd = not detectable; -* = not reported

Table 10: Identified metabolites (% administered dose) in urine of rats 24-168 h after oral administration of ^{14}C-BAS 800 H at 100 mg/kg bw.

biosample	Urine									Feces								
sex	♂					♀				♂					♀			
Assessed 0-h	-24		-96		-168	-24	-96	-168		-24		-72	-96		-24	-48	-72	
Radio-label	P	U	P	U	P	P	P	P	U	P	U	P	P	U	P	P	P	U
BAS 800 H	21.7	20.9	23.1	22.2	36.6	43.9	48.7	82.1	53.3	14.6	13.7	4.84	16.0	15.2	7.54	3.75	9.94	9.20
M800H01	2.95	3.39	3.53	3.93	9.05	nd	0.06	0.47	0.05	13.2	12.9	23.0	22.3	25.8	0.52	1.16	0.98	1.27
M800H05	0.50	0.29	0.69	0.44	1.76	-	-	nd	-	0.29	nd	0.77	0.59	0.33	-	0.18	-	-
M800H03	0.59	0.63	0.75	0.81	2.21	nd	0.04	0.05	-	1.66	1.52	2.40	2.65	2.71	0.15	0.65	0.36	0.45
M800H07	0.28	-*	0.40	-	2.16	0.42	0.63	3.64	-	0.85	-	1.41	1.20	-	0.30	0.75	0.76	-
M800H37	-	-	0.02	-	0.19	-	-	0.01	-	0.47	-	1.79	0.83	-	-	0.10	-	-
M800H02+06	nd	nd	nd	nd	-	nd	nd	-	-	1.32	1.13	2.90	2.68	3.20	nd	0.32	0.16	0.36
M800H09	-	0.30	-	0.34	-	-	-	0.01	0.01	-	nd	-	-	nd	-	-	-	-
M800H04+08	nd	nd	nd	nd	-	nd	nd	-	-	0.97	1.52	0.95	1.38	2.37	0.61	0.59	1.24	0.78
M800H23	-	0.26	-	0.38	-	-	-	-	0.50	-	nd	-	-	nd	-	-	-	-
total	26.0	25.8	28.5	28.1	52.0	44.3	49.5	86.3	53.4	33.3	30.8	38.1	47.6	50.6	9.12	7.50	13.4	12.1

Data taken from Tables 14-17, 22-26, pages 65-67, 70-72 of Metabolism Report; nd = not detectable; -* = not reported

Metabolites in bile: Table 11

The excretion of radioactivity via bile amounted to 19-68% AD within 48 h for male and female rats.

For male rats, the major metabolites were M800H01 (8.7-10.6% AD), M800H07 (11.1-13.4% AD), M800H18 (8.9-11.5% AD), and the unchanged parent compound (4.8-14.5% AD). The metabolite M800H18 was identified as a derivative of the parent compound in which the uracil ring was cleaved to form a N-sulfonylamide group, combined with a demethylation of the N-methyl-N-isopropylgroup. Other metabolites identified in the bile were M800H02 + M800H17 (2.1-4.4% AD), M800H16 (1.6-2.4% AD), M800H19 (3.1-3.2% AD), M800H20 (4.1-4.6% AD), and M800H21 (0.2% AD). M800H17 was identified as a derivative of the parent compound in which the uracil ring was cleaved but the three-carbon fragment remained attached to the molecule. M800H20 and M800H19 were formed by further degradation of the N-methyl-N-isopropylgroup. Metabolite M800H16 could be formed by oxidation of

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M800H17. M800H21 was an oxidation product of BAS 800 H.

In female rats, the predominant biliary metabolites were M800H07 (4.8-8.7% AD) and the parent compound (6.6-11.4% AD). Other identified minor metabolites were M800H01 (0.7-1.1% AD), M800H02 + M800H17 (1.7-3.1% AD), M800H16 (0.8-1.7% AD), M800H18 (0.6-1.1% AD), M800H19 (0.8-2.1% AD, M800H20 (0.7-1.4%), and M800H21 (0.1-0.3% AD).

Total identified metabolites in bile were in the range of 47-62 and 17-31% AD in male and female rats, respectively.

Table 11: Identified metabolites (% administered dose) in the rat 48-72 h after oral administration of ^{14}C-BAS 800 H at 5 or 100 mg/kg bw.

phenyl-^{14}C-BAS 800 H	5 mg/kg bw		100 mg phenyl-^{14}C-BAS 800 H/kg bw	
administration	single		single	
bio-sample	bile		bile	
sex	♂	♀	♂	♀
h assessed	0-48	0-48	0-48	0-48
BAS 800 H	4.78	6.56	14.5	11.4
M800H18	11.5	0.61	8.87	1.14
M800H07	11.1	4.77	13.4	8.08
M800H01	8.69	0.72	19.6	1.13
M800H20	4.11	0.70	4.58	1.43
M800H13	3.06	-	-	-
M800H02+17	2.08	1.75	4.42	3.08
M800H16	1.62	0.82	2.43	1.67
M800H21	0.17	0.14	0.26	0.32
M800H19	-	0.83	3.21	2.06
total	47.0	16.9	62.2	30.9
Data taken from Tables 27-30, pages 73-76 of Metabolism Report; -[a] = not reported;				

Metabolites in tissues: Tables 12-13

Table 12: Identified metabolites (% administered dose) in the rat 1 h after oral administration of phenyl- and uracil-^{14}C-BAS 800 H at 5 mg/kg bw.

	♂								♀							
tissue	liver		kidneys		fat		plasma		liver		kidneys		fat		plasma	
compound	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
BAS 800 H	25.4	19.1	0.77	0.66	0.72	0.50	2.73	3.53	16.4	15.7	0.63	0.71	0.61	0.69	3.38	4.10
M800H04	4.30	2.88	0.24	0.21	nd	0.03	nd	nd	1.61	2.46	0.14	0.24	nd	0.02	nd	0.17
M800H01	1.32	1.09	0.03	0.02	0.03	0.01	0.03	nd	nd	0.21	nd	0.01	nd	nd	nd	nd
M800H03	0.15	nd	0.01	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
total	31.2	23.1	1.04	0.90	0.73	0.54	2.76	3.53	18.0	18.4	0.77	0.96	0.61	0.71	3.38	4.27
Data taken from Tables 31and 33, pages 77 and 78 of Metabolism Report; nd = not detectable A = phenyl-^{14}C-BAS 800 H; B = uracil-^{14}C-BAS 800 H																

Table 13: Identified metabolites (% administered dose) in the rat 1 h after oral administration of phenyl- and uracil-^{14}C-BAS 800 H at 100 mg/kg bw.

	♂								♀							
tissue	liver		kidneys		fat		plasma		liver		kidneys		fat		plasma	
compound	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
BAS 800 H	4.34	7.28	0.44	0.64	0.23	0.38	1.57	1.97	6.07	4.64	0.72	0.47	0.74	0.46	2.46	2.01
M800H04	1.29	2.10	0.14	0.23	0.01	0.02	0.03	0.06	1.36	1.31	0.29	0.23	nd	0.01	nd	0.05
M800H01	0.15	0.24	0.02	0.02	0.0	nd	nd	nd	nd	nd	nd	0.01	nd	nd	nd	nd
M800H03	nd	nd	0.00	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
M800H07	nd	nd	0.01	nd	nd	nd	nd	nd	nd	nd	0.01	nd	nd	nd	0.04	nd
total	5.80	9.62	0.61	0.89	0.24	0.49	1.60	2.03	7.43	5.95	1.03	0.70	0.74	0.47	2.50	2.06
Data taken from Tables 32 and 34, pages 77-78 of Metabolism Report; nd = not detectable A = phenyl-^{14}C-BAS 800 H; B = uracil-^{14}C-BAS 800 H																

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The metabolites in the liver, kidneys, and adipose tissues were comparable. The major metabolites were the unchanged parent compound (liver = 4.4-25.4, kidneys = 0.4-0.8, fat = 0.2-0.7% AD) and M800H04 (liver = 1.3-4.3, kidneys = 0.1-0.3, fat <0.01-0.03% AD). Other minor metabolites were M800H01 and M800H03. Total identified metabolites in the liver, kidneys, and fat were 6.0-31, 0.8-1.0, and 0.2-0.7% AD, respectively. Overall, no considerable effects were detectable between the genders in tissues.

Metabolites in plasma:

The metabolites in the plasma were also comparable to those in the tissues such as the liver and kidneys. The predominant metabolites were the unchanged parent compound (1.6-4.1% AD), M800H04 (0.01-0.06% AD). The metabolite M800H01 was identified only in male rats (phenyl-label) at 0.03% AD, while M800H07 was only seen in female rats (phenyl-label) at 0.04% AD. Total identified metabolites in plasma were in the range of 1.6-4.3 % of the dose.

Metabolic pathway: The identified metabolites are summarized in Table 14.

Table 14: Identified metabolites in the rat after oral administration of phenyl- and uracil-^{14}C-BAS 800 H

metabolite	urine		feces		bile	liver		kidneys		fat		plasma	
^{14}C-BAS 800 H	A	B	A	B	A	A	B	A	B	A	B	A	B
BAS800H	x	x	x	x	x	x	x	x	x	x	x	x	x
M800H01	x	x	x	x	x	x	x	x	x	x	x	x	
M800H02	x	x	x	x	x								
M800H03	x	x	x	x		x		x					
M800H04			x	x		x	x	x	x	x	x	x	x
M800H05	x	x	x	x									
M800H06	x	x	x	x									
M800H07	x		x	x	x			x					
M800H08			x	x									
M800H09	x	x	x										
M800H010		x											
M800H011	x		x		x								
M800H016					x								
M800H017					x								
M800H018					x								
M800H019					x								
M800H020					x								
M800H021					x								
M800H023		x											
M800H035					x								
M800H037	x		x										
Data taken from Table 35, pages 79-80 of Metabolism Report; A = phenyl^{14}C-BAS 800 H; B = uracil^{14}C-BAS 800 H													

In rats, BAS 800 H was metabolized by three major transformation steps:
- demethylation of the uracil ring
- degradation of the N-methyl-N-isopropylgroup to NH_2
- cleavage of the uracil ring, forming a sulfonylamide group

The predominant metabolites were M800H01, M800H03, M800H07, and the parent compound. In addition, the metabolites, M800H05 and M800H18 also occurred in male rats.

The metabolites M800H01, M800H03, and M800H05 were identified as derivatives of BAS 800 H in which the N-methyl-N-isopropylgroup was step-by-step degraded to unsubstitutes sulfonamide. The metabolite M800H07 was identified as a derivative of the parent compound in which the uracil ring was cleaved with loss of three carbons to form a phenyl-N-methyl urea group. The metabolite M800H18 was identified as a derivative of the parent compound in which the uracil ring was cleaved to form a phenyl-N-methyl urea, combined with a demethylation of the N-methyl-N-isopropyl sulfonamide.

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Other identified metabolites were M800H02, M800H04, M800H06, M800H08, M800H09, M800H10, M800H11, M800H16, M800H17, M800H19, M800H20, M800H21, M800H23, M800H35, and M800H37.

The metabolic pathway is shown in Figure 1.

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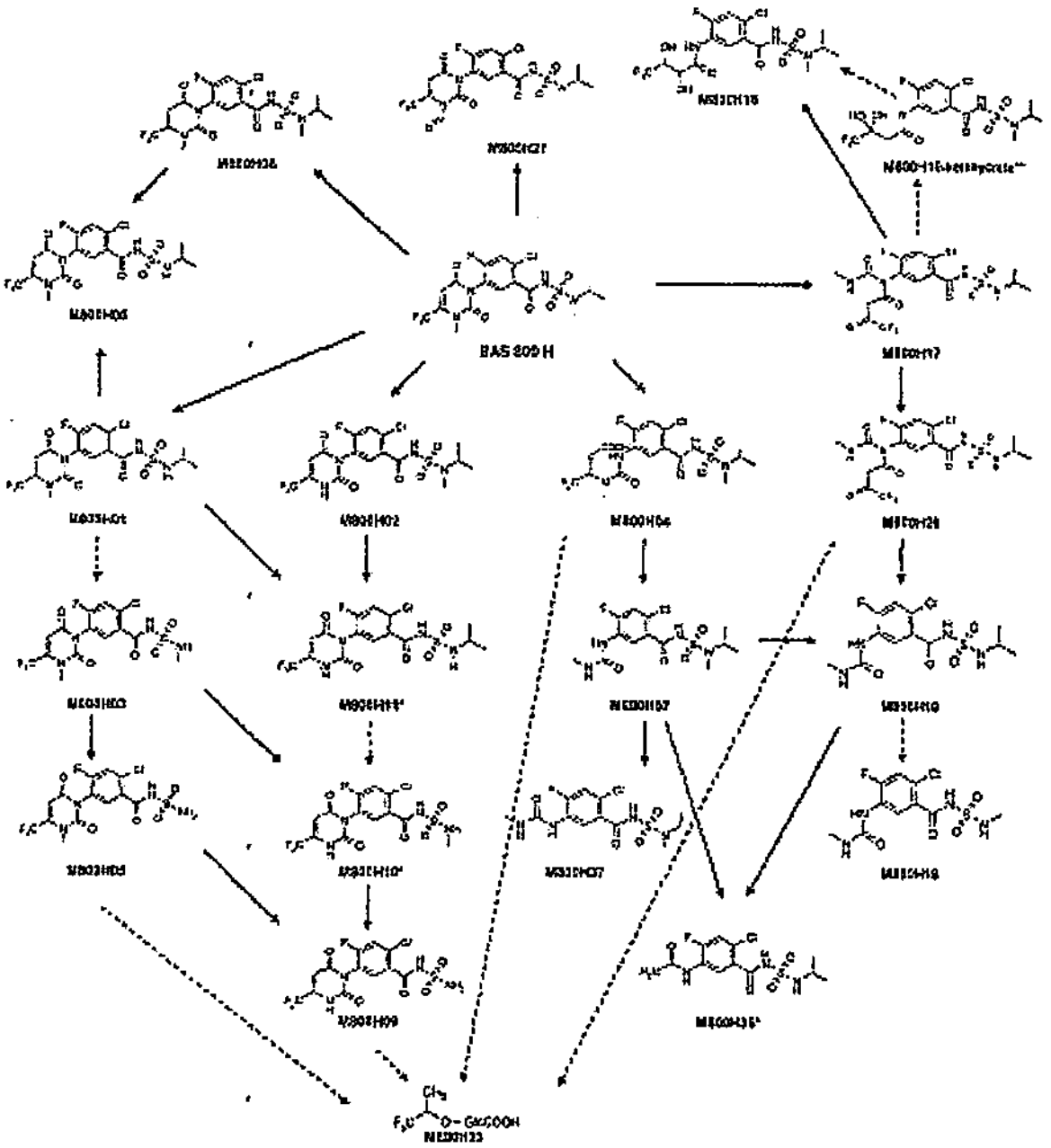
Figure 1: Metabolic pathway of BAS 800 H in rats



Remarks: *: Metabolites were identified by LC-MS/MS. Quantification within the study was not feasible, because of negligible amounts.
** Metabolite M800H15-ketohydrate is a potential intermediate metabolite not identified during the current study

III. DISCUSSION

A. Authors' conclusions:

"After single oral administration, ^{14}C-BAS 800 H was rapidly absorbed from the gastrointestinal tract. Based on the amounts of radioactivity excreted via bile and urine, the bioavailability of ^{14}C~BAS 800 H in male rats was virtually complete at a dose of 100 mg/kg bw and was calculated to be about 70 % at a dose level of 5 mg/kg bw. For female rats, the applied dose of ^{14}C-BAS 800 H was virtually completely absorbed at both dose levels. For the assessment of the calculated bioavailability, it has to be taken into account that the data indicate potential re-absorption of at least a part of the biliary excreted radioactivity. The excretion of radioactivity was rapid and occurred sex and dose specific with a higher urinary excretion in female rats that was more pronounced at lower doses. Thus, the clearance of ^{14}C-BAS 800 H was higher in female rats with the consequence that female rats have lower internal exposures than male rats at the same external dose.

The parent compound was metabolized by three major transformation steps, which are demethylation of the uracil ring system, degradation of the N-methyl-N-isopropylgroup to NH_2 and cleavage of the uracil ring, forming a sulfonylamide group. The predominant metabolites were M800H01, M800H03, M800H07 and the parent compound for male and female rats. In addition, the metabolite M800H05 was a major metabolite for male rats. Metabolites M800H16, M800H17, M800H18, M800M19 and M800M20 were present in bile only at remarkable portions."

B. Reviewer's comments:

The study was properly conducted and reported. The biokinetics data demonstrated that orally administered BAS 800 H was rapidly absorbed, distributed, and excreted. Regardless of the dose administered, maximum concentration of BAS 800 H in blood and plasma was reached within 1 h of dosing and declined rapidly after 24 h. The blood and plasma data demonstrated that the majority of the BAS 800 H residues occurred in the plasma and were not bound to cellular elements of the blood. There was a sex dependent difference in the excretion of orally administered BAS 800 H. Following single low- and high-dose administration or a repeat high-dose administration, the main route of elimination in male rats was via the feces, while urinary excretion was the major route of elimination in females. The sex dependent excretion was more pronounced at the low dose level than at the high dose level. The sex dependent difference in excretion of orally dosed BAS 800 H was also demonstrated by the biliary excretion data which showed significantly higher biliary excretion of BAS 800 H residues in males than in females. Excretion of orally dosed BAS 800 H was essentially complete within 96 h, the majority was eliminated within the first 24 to 48 h. Exhalation was not a relevant excretion pathway of BAS 800 H. At 168 h after dosing, BAS 800 H residues remaining in tissues were very low, and occurred mainly in carcass, liver, skin, and gut contents.

Studies of the metabolic pattern and identification of metabolites in the rats indicated that BAS 800 H was metabolized by three major transformation steps, which were demethylation of the uracil ring system, degradation of the N-methyl-N-isopropylgroup to NH_2, and cleavage of the uracil ring, forming a sulfonylamide group. The predominant metabolites were M800H01, M800H03, M800H07 and the parent compound. Other minor metabolites were M800H05, M800H16, M800H17, M800H18, M800M19, and M800M20. There were no significant gender differences in metabolic profiles.

Both the biokinetics and metabolism studies were well conducted. However, reporting of the results was difficult to follow because of the many dose groups tested and the large amount of data collected.